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BY

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ELFRIDA CONSTANCE VICTORIA VENN

[From THE BIOCHEMICAL JOURNAL, Vol. XIV, Nos. 3 & 4, July, 1920]

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XXXVI. THE ACIDITY OF ROPY MILK.

By KATHLEEN FREEAR

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Ropy Milk.

In 1916 two samples of ropy milk from two Lancashire farms were sent to the laboratory by Miss Stubbs, Lancashire County Council Dairy School, Hutton.

The samples were plated out upon neutral lemco agar and from both of them a gram-positive *Coccus* showing also diplococcal forms, and in fluid cultures short chains, was obtained. Sometimes the cocci appeared lanceolate and possibly capsulated, the latter appearance being most marked in glucose broth cultures. The cultures which were obtained were called *Bury* and *Accrington* since the farms from which the samples came were in the neighbourhoods of these towns.

After satisfactory isolation had been carried out the bacteriological characteristics of two strains B_{12} and B_{15} from the Accrington farm and two others RM_4 and RM_5 from the Bury farm were studied. The results obtained appeared to show that these organisms were either identical with or closely related to the *Streptococcus Hollandicus*, and they were used as starters with satisfactory results in the preparation of Edam cheese.

For purposes of comparison strains of *Streptococcus Hollandicus* were obtained from Prof. Orla Jensen and also from Hoorn and Delft. The reactions of these strains were studied upon solutions of sugars, alcohols, etc. which were prepared in the following way. Tubes of media containing 1 % peptone and 0·5 % NaCl were neutralised to litmus; they were then coloured with litmus and 0·5 % of the "sugar" was added except in the cases of inositol, sorbitol and dulcitol, of which only 0·25 % was used. The tubes were then sterilised by steaming on three consecutive days. The reactions produced by the various strains upon these media are shown in Table I. It does not appear that the strains which had been isolated from the milk obtained from Accrington and Bury differed in any material respect from those found with the strains of *Streptococcus Hollandicus*, especially when the method of preparation of the media, and the difficulty of obtaining constant reactions with streptococci upon such media are considered.

Table I.

Ropy Milk.
February 21, 1917.

	Accrington	Bury	Hoorn	Delft	Jensen
Saccharose	—	Sl. acid	—	—	?
Mannitol	Acid	Acid	—	—	V. sl. acid
Galactose	Acid	Acid	Acid	—	—
Adonitol	—?	—	—	—	?
Dextrose	Acid	Acid	Acid	Sl. acid	?
Glycerol	Sl. acid?	—	—	—	?
Dextrin	Acid	Acid	Acid	—	—
Raffinose	—	—	—	—	—
Lactose	Acid	Acid	Acid	—	—
Inulin	Sl. acid?	V. sl. acid?	—	—	?
Sorbitol	—?	—	?	—	—
Inositol	—?	—	V. sl. acid	?	Sl. acid
Dulcitol	—?	Acid	—	?	?
Salicin	Acid	V. sl. acid?	Acid	—	Acid?

While the bacteriological examinations of the strains were being carried out an occasional determination of the acidity of samples of the ropy milk was made, and was always found to fall within narrow limits. It was therefore decided to study the range of acidity within which the isolated strains produced ropiness and with this object the following experiments were carried out.

The milk under investigation was said to be "Ropy," when definite threads could be drawn out from its surface by means of a sterile platinum needle. Under the conditions of experiment very long ropy threads were not obtained and the ropiness was considered to be "*good*" when the length of the threads approximated to an inch. When the threads were considerably shorter than this but nevertheless distinguishable from mere stickiness, the ropiness was described as "slight." During the last series of determinations the degree of ropiness was further estimated by means of simple viscosity determinations. The method was not sufficiently accurate to justify the inclusion of the results in the text, but it may be mentioned that simultaneously with the appearance of "good rope" there was a sudden and very marked increase in viscosity. With the diminution of "ropiness" there was a corresponding decrease in viscosity, until with its disappearance the viscosity value obtained closely approximated to that of the uninoculated milk.

In the first series of experiments, the results of which are shown in Fig. 1, flasks of sterile milk, which had been inoculated with the strains isolated from the samples of ropy milk, were incubated at 22°. Samples were withdrawn, tests for ropiness made, and determinations of acidity with $N/9$ NaOH using phenolphthalein as indicator were carried out every 24 hours for a period of 7 days. Fig. 1 shows that ropiness was found on the first day after inoculation except in the case of B_{15} when it did not appear until the second day, and that it occurred between acidities which varied between 0·44 and 0·75 (% lactic acid calculated from titrations with $N/9$ NaOH). Ropiness was well marked

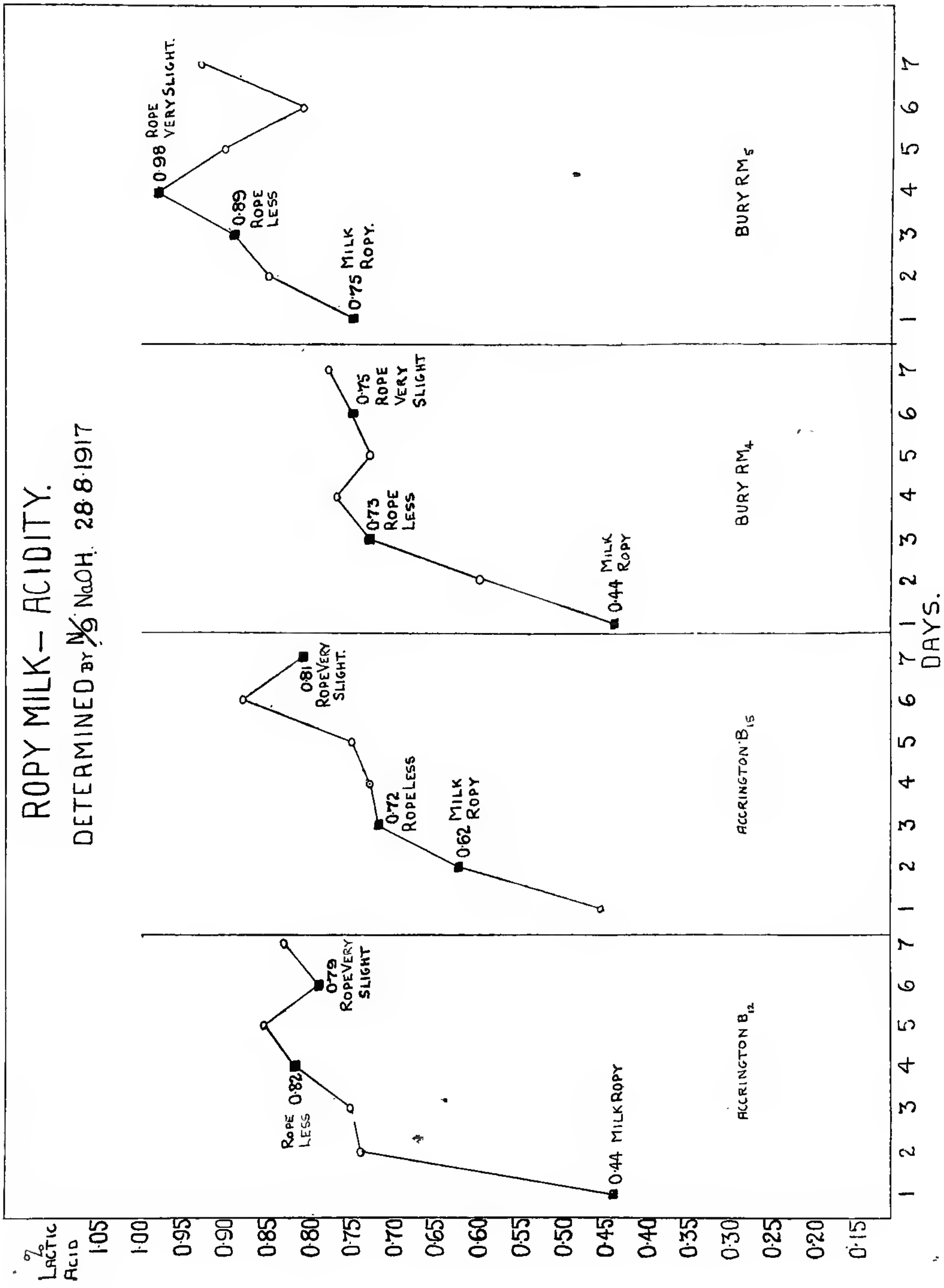
ROPY MILK— ACIDITY.
DETERMINED BY N/9 NaOH. 28.8.1917
% Lactic Acid
1·05
1·00
0·95
0·90
0·85
0·80
0·75
0·70
0·65
0·60
0·55
0·50
0·45
0·40
0·35
0·30
0·25
0·20
0·15
DAYS.
1 2 3 4 5 6 7
Accrington B$_{12}$
0·44 Milk Ropy
Rope Less 0·82
0·79 Rope Very Slight
Accrington B$_{15}$
0·62 Milk Ropy
0·72 Rope Less
0·81 Rope Very Slight.
Bury RM$_4$
0·44 Milk Ropy
0·73 Rope Less
0·75 Rope Very Slight
Bury RM$_5$
0·75 Milk Ropy.
0·89 Rope Less
0·98 Rope Very Slight.

Fig. 1.

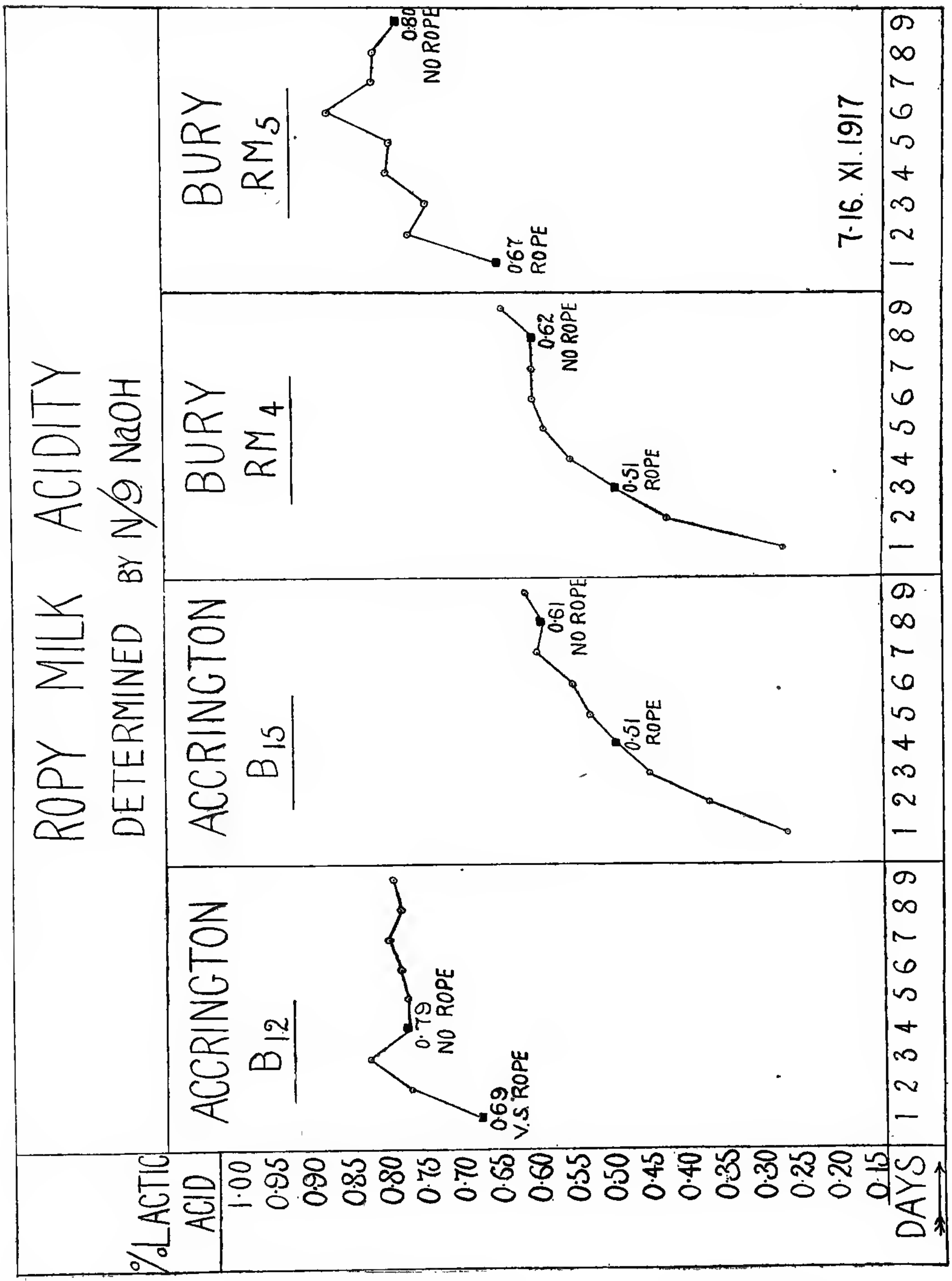
ROPY MILK ACIDITY
DETERMINED BY N/9 NaOH
ACCRINGTON B12
ACCRINGTON B15
BURY RM 4
BURY RM5
0·69 V.S. ROPE
0·79 NO ROPE
0·51 ROPE
0·61 NO ROPE
0·51 ROPE
0·62 NO ROPE
0·67 ROPE
0·80 NO ROPE
7-16. XI. 1917
% LACTIC ACID
1·00
0·95
0·90
0·85
0·80
0·75
0·70
0·65
0·60
0·55
0·50
0·45
0·40
0·35
0·30
0·25
0·20
0·15
DAYS
1 2 3 4 5 6 7 8 9

Fig. 2.

for the first three or four days, at the end of which time the acidities varied between 0·72 and 0·89; subsequently the ropiness diminished without any marked increase of acidity, but was still present to a very slight extent at the end of the experiments. A second series of experiments was then undertaken, partly to confirm the results already obtained, and partly to prolong the duration and, if possible, to find the period at which the ropiness really disappeared. Fig. 2 shows that sometimes ropiness did not appear until the fourth day after inoculation. It continued to be present in gradually diminishing amount for periods which varied from three to eight days. The range of acidity over which ropiness was present was 0·51 to 0·87. At the end of the experiments the ropiness had disappeared, though no appreciable alteration in the acidity had taken place.

In continuation of this series two strains from each of the cultures which were under investigation were studied and with them two controls of sterile inoculated milk. Fig. 3 shows that the acidities of the controls lay within the values 0·17 and 0·20. In all the inoculated flasks ropiness was found at the end of 24 hours, the acidity varying at this time from 0·59 to 0·82. The ropiness remained well marked for periods which varied from one to three days with acidities which lay between 0·59 and 0·96 except upon one occasion when the acidity was found to be 1·435. It is doubtful whether this reading does not represent an experimental error. The ropiness began to diminish from two to four days after inoculation and became gradually less until it ceased to be present on the sixth to the ninth day. In all cases the ropiness had disappeared at the end of the experiment which was carried out over a period of nine days. The diminution of ropiness was accompanied by an acidity which varied between 0·83 and 1·0. The disappearance of ropiness was not accompanied by any material variation in the acidity.

In these experiments the examinations of the samples were first made 24 hours after inoculation of the milk, by which time the ropiness was in many cases well established. In all later experiments, therefore, the inoculations were made at midnight and the milk was tested at six hour intervals for the first 18 hours, and afterwards at intervals of 12 hours. The third series of experiments was carried out under these conditions and Fig. 4 shows that at the 30th hour after inoculation the acidity was 0·30 and no ropiness was found, but at the 42nd hour the acidity was 0·73 with fair ropiness. At the end of another 12 hours, when the acidity was 0·9 the ropiness was already decreasing, and continued to decrease until it had completely disappeared 102 hours after the inoculation when the acidity was 0·97. The highest acidity recorded during the period of the experiment was 1·00.

It was thought that the initial acidity at which ropiness occurred might be fixed with greater accuracy if more continuous determinations were made, but it was not possible to carry out satisfactory titration experiments at night. In the later experiments, therefore, the acidity determinations were made not only by titrating with $N/9$ NaOH but also electrometrically.

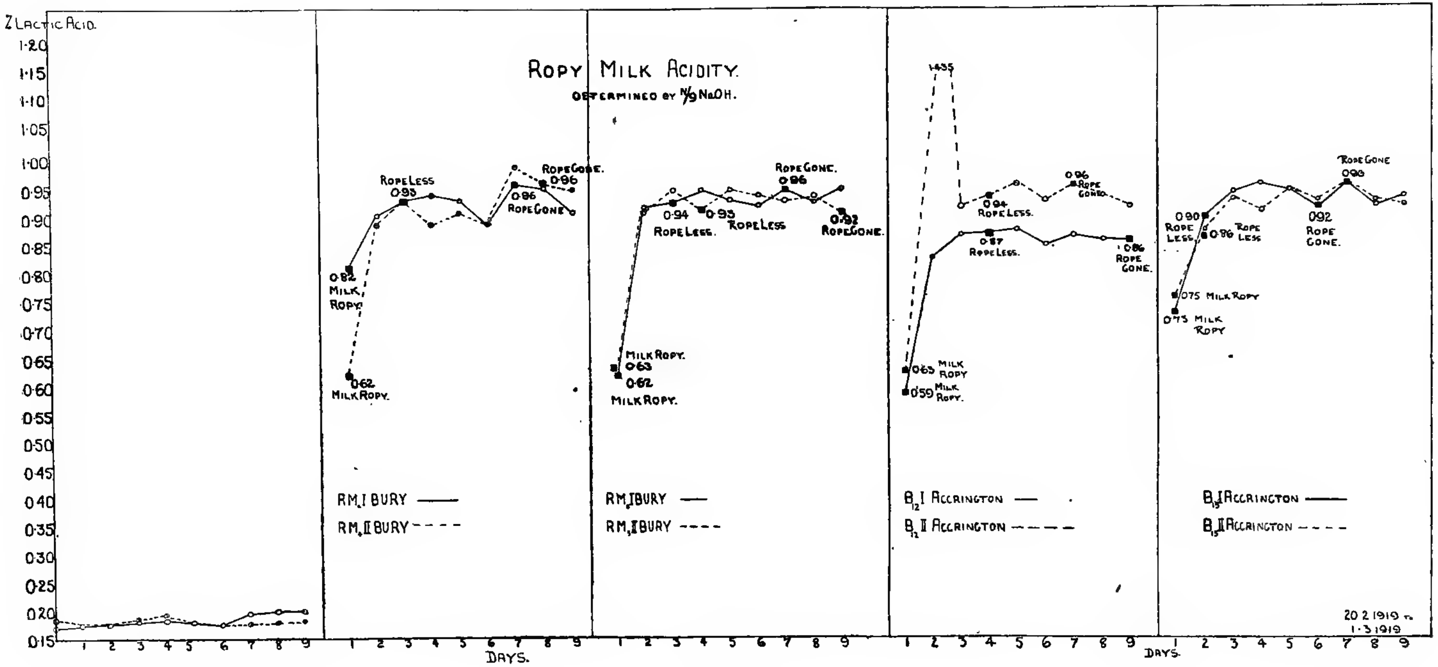
Ropy Milk Acidity.
Determined by N/9 NaOH.
% Lactic Acid.
1·20
1·15
1·10
1·05
1·00
0·95
0·90
0·85
0·80
0·75
0·70
0·65
0·60
0·55
0·50
0·45
0·40
0·35
0·30
0·25
0·20
0·15
1 2 3 4 5 6 7 8 9
Days.
0·82 Milk, Ropy.
0·62 Milk Ropy.
Rope Less 0·93
Rope Gone. 0·96
0·86 Rope Gone.
RM I Bury
RM II Bury
Milk Ropy. 0·63
0·62 Milk Ropy.
0·94 Rope Less.
0·93 Rope Less
Rope Gone. 0·96
0·92 Rope Gone.
RM I Bury
RM II Bury
1·435
0·65 Milk Ropy
0·59 Milk Ropy.
0·94 Rope Less.
0·87 Rope Less.
0·96 Rope Gone.
0·86 Rope Gone.
B12 I Accrington
B12 II Accrington
0·90 Rope Less.
0·86 Rope Less
0·75 Milk Ropy
0·73 Milk Ropy
0·92 Rope Gone.
Rope Gone 0·96
B15 I Accrington
B15 II Accrington
20 2 1919 to 1·3 1919

Fig. 3.

Figs. 5 and 6 show the results obtained by both methods in the course of the next experiments. It is seen that ropiness was present at the 18th hour after inoculation, when the percentage of lactic acid was 0·43 and the P_H was 5·0. It continued to be well marked for 102 hours, when it was found to be diminishing. During this period the acidity as determined by $N/9$ NaOH rose from 0·43 to 0·81, and the P_H changed from 5·0 to 4·0. After the 102nd hour the ropiness diminished, while the acidity remained practically unchanged. The quantity of milk available was insufficient to carry on the experiment beyond 138 hours, and it was therefore repeated with a much larger bulk of milk. In order to ensure sterility of such a volume the heating was necessarily prolonged with the result that a certain amount of charring took place, rendering the end point of the titrations with $N/9$ NaOH indeterminate, for which reason these readings are not included. The results of the electrometric determinations are shown in Fig. 7 from which it appears

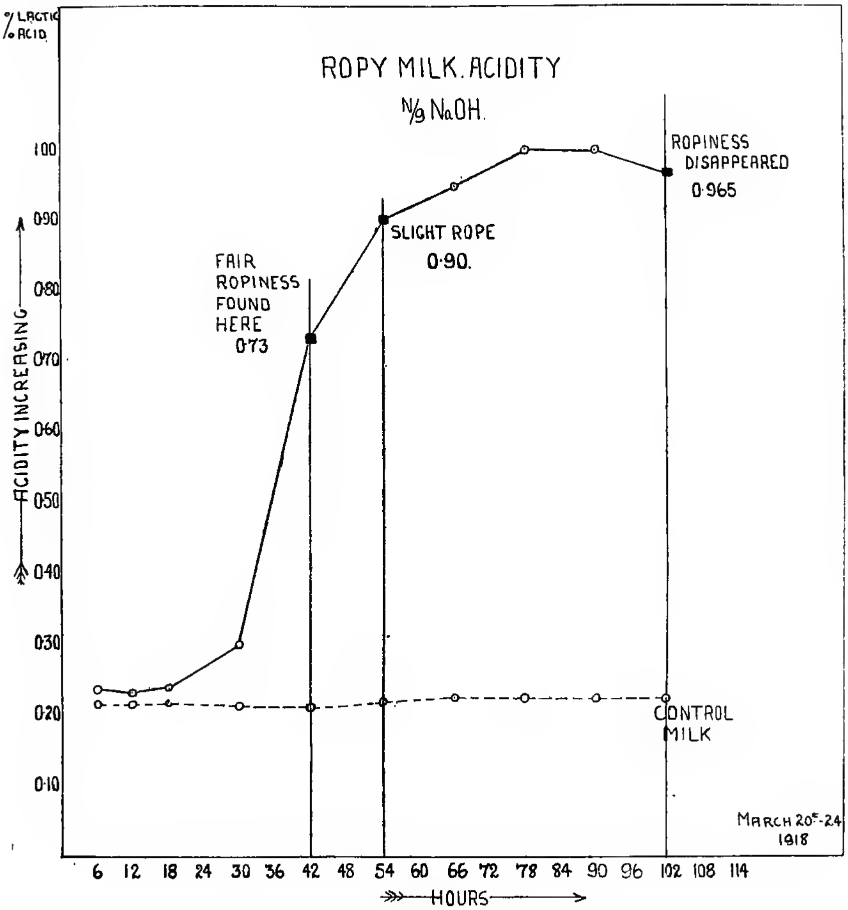

Fig. 4.

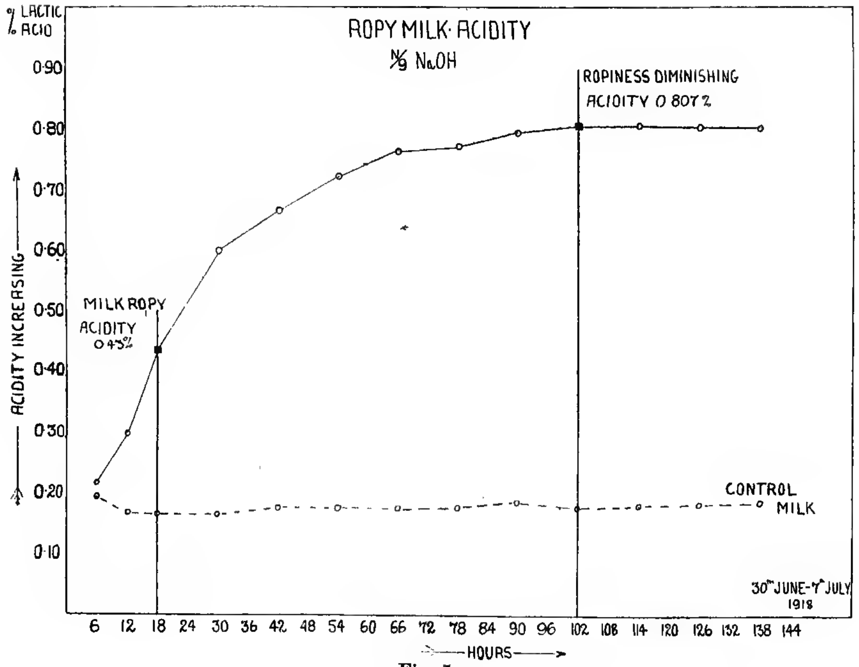
% LACTIC ACID
ROPY MILK ACIDITY
N/9 NaOH
0·90
0·80
0·70
0·60
0·50
0·40
0·30
0·20
0·10
ACIDITY INCREASING
MILK ROPY
ACIDITY 0·43%
ROPINESS DIMINISHING
ACIDITY 0·807%
CONTROL MILK
30th JUNE-7th JULY 1918
6 12 18 24 30 36 42 48 54 60 66 72 78 84 90 96 102 108 114 120 126 132 138 144
HOURS

Fig. 5.

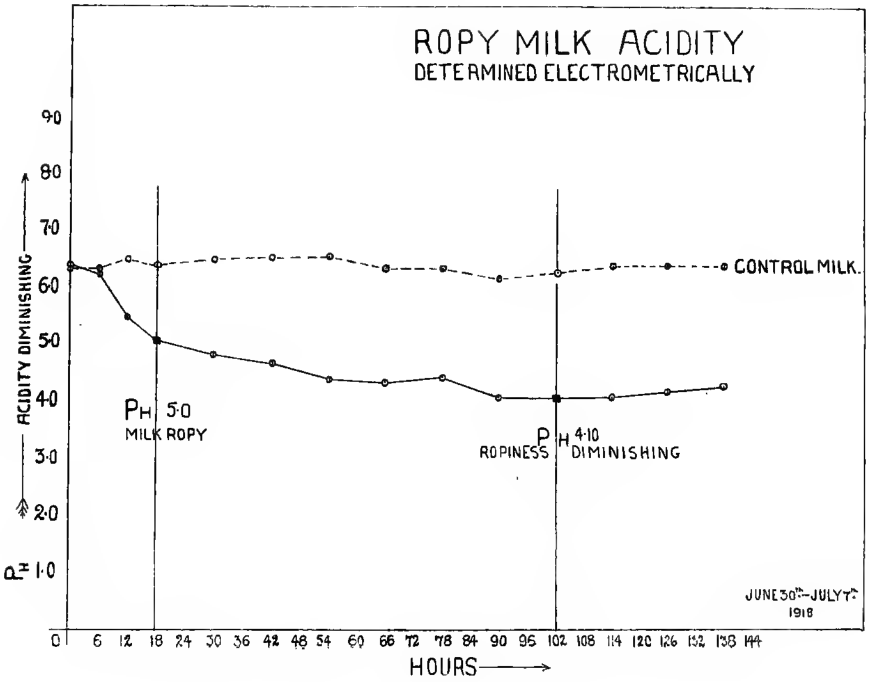
ROPY MILK ACIDITY
DETERMINED ELECTROMETRICALLY
9·0
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
ACIDITY DIMINISHING
PH 5·0
MILK ROPY
PH 4·10
ROPINESS DIMINISHING
CONTROL MILK.
JUNE 30th–JULY 7th 1918
0 6 12 18 24 30 36 42 48 54 60 66 72 78 84 90 96 102 108 114 120 126 132 138 144
HOURS

Fig. 6.

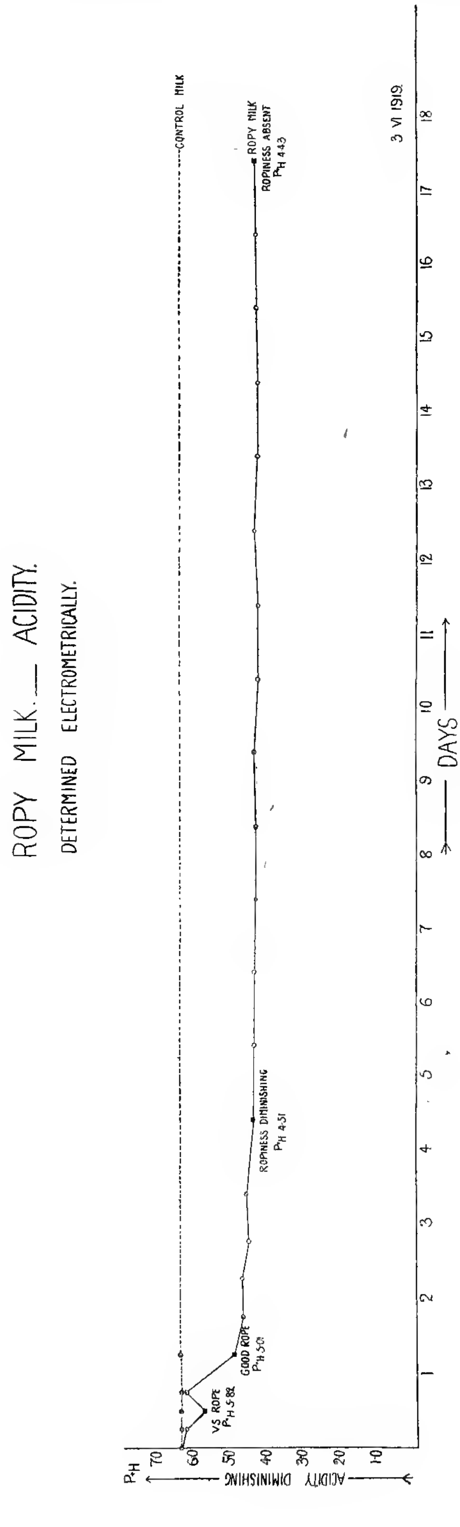
ROPY MILK.— ACIDITY.
DETERMINED ELECTROMETRICALLY.
CONTROL MILK
ROPY MILK
ROPINESS ABSENT
PH 4·43
3 VI 1919
ROPINESS DIMINISHING
PH 4·51
GOOD ROPE
PH 5·01
VS ROPE
PH 5·82
PH
ACIDITY DIMINISHING
70
60
50
40
30
20
1·0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
DAYS

Fig. 7.

that slight ropiness was first found at the 12th hour after inoculation when the P_H was 5·82. At the 30th hour good ropiness had developed and the P_H was 5·01. Satisfactory ropiness continued for three days at the end of which time the P_H was 4·51. The ropiness then gradually diminished until, 17 days after the inoculation, it had entirely disappeared, but throughout this period the acidity remained practically unchanged.

CONCLUSIONS.

The experiments show that these strains produced ropiness in milk, associated with a minimum acidity, as determined by $N/9$ NaOH, of 0·43 % lactic acid, and that the ropiness continued for varying periods of time, accompanied by an acidity which never rose above 1·0 % (except on one occasion). In every case, the ropiness eventually diminished and when studied for sufficiently long periods entirely disappeared, but these changes were not accompanied by any material alteration in the acidity.

The results obtained by the NaOH titration method were confirmed by electrometric determinations, from which it appeared that ropiness was found in milk with a P_H range of 5·82 to 4·10. With diminishing ropiness no appreciable alteration in the P_H value could be detected, although in one case the experiment was continued for 17 days by which time ropiness had disappeared.

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